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Cork Layer Development and Cuticle Thickening in Stems of the Genus *Magnolia* Subgenus Yulania

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Cork layer development and cuticle thickening were examined in stems of the genus *Magnolia* subgenus Yulania. Three types of stem anatomy were distinguishable. The first type found in *M. acuminata, M. heptapeta, M. quinquepeta* and *M. tomentosa* is characterized by the rapid development of a cork layer between cortex and epidermis and the thin cuticle. The second type is observed only in *M. praecocissima.* In this species cork layer development is rather slow and the cuticle becomes thick. The third type found in *M. salicifolia* (and presumably in *M. biondii*) is characterized by very slow development of a cork layer and by a thick cuticle.

Key words: *Magnolia*, stem, anatomy, cork layer, cuticle

The genus *Magnolia* subgenus Yulania is composed of Sect. Yulania, Sect. Buergeria and Sect. Tulipastrum (Dandy 1978). Floral buds of many species attributed to this subgenus, such as *M. amoena* Cheng, *M. biondii* Pamp., *M. heptapeta* (Buchoz) Dandy (= *M. denudata* Desr.), *M. praecocissima* Koidz. (= *M. kobus* DC.), *M. quinquepeta* (Buchoz) Dandy (= *M. liliflora* Desr.), *M. salicifolia* (Maxim.) Siebold & Zucc., *M. sprengeri* Pamp. and others are called Xinyi（辛夷）and have been used mainly for treatment of inflammation and pain in traditional Sino-Japanese medicine (Song 1984, Ministry of Health and Welfare 1989, Institute of Materia Medica Chinese Academy of Medicinal Sciences et al. 1994, Namba 1994, Chinese Pharmacopoeia Committee of Ministry of Public Health of the People's Republic of China 1995). Pharmacognostic studies on Xinyi were made by Kimura et al. (1961), Zhao et al (1986), and Wu et al (1991), and it was revealed that floral buds of *Magnolia* species used as Xinyi are distinguishable from each other by anatomical features. Terabayashi and Okada (1991) examined the anatomy of stems bearing floral buds. The results given here are that cuticle thickness in the stems beneath floral buds varies among species and that cork layers are present in some species but absent in others. In order to gain a better understanding of variation of stem anatomy in the genus *Magnolia* subgenus Yulania, it is highly necessary to investigate the stem anatomy from a developmental viewpoint. In this article cork layer development and cuticle thickening were observed in several species of subgenus Yulania and compared among the species.

Materials and Methods

Sources of plant material used in this

Table 1. *Magnolia* species used in this study

Species	Individual No.	Source	Voucher
Magnolia acuminata	1	Cult. Ushiku, Ibaraki	Terabayashi 1342
	2	Cult. Ushiku, Ibaraki	Terabayashi 1343
M. biondii	1	Cult. Ami, Ibaraki	Terabayashi 1411
	2	Cult. Ami, Ibaraki	Terabayashi 1412
M. heptapeta	1	Cult. Ushiku, Ibaraki	Terabayashi 1209
(= *M. denudata*)	2	Cult. Ami, Ibaraki	Terabayashi 1207
	3	Cult. Ami, Ibaraki	Terabayashi 1208
M. praecocissima	1	Cult. Ushiku, Ibaraki	Terabayashi 1345
(= *M. kobus*)	2	Cult. Ushiku, Ibaraki	Terabayashi 1344
	3	Cult. Ami, Ibaraki	Terabayashi 1346
	4	Cult. Ami, Ibaraki	Terabayashi 1115
	5	Cult. Amakubo, Tsukuba, Ibaraki	Terabayashi 1356
M. quinquepeta	1	Cult. Ushiku, Ibaraki	Terabayashi 1350
(= *M. liliflora*)	2	Cult. Ushiku, Ibaraki	Terabayashi 1349
	3	Cult. Ami, Ibaraki	Terabayashi 1203
	4	Cult. Ami, Ibaraki	Terabayashi 1204
M. salicifolia	1	Cult. Amakubo, Tsukuba, Ibaraki	Terabayashi 1113
	2	Cult. Amakubo, Tsukuba, Ibaraki	Terabayashi 1114
M. tomentosa	1	Cult. Ushiku, Ibaraki	Terabayashi 1347
(= *M. stellata*)	2	Cult. Ushiku, Ibaraki	Terabayashi 1111
	3	Cult. Ushiku, Ibaraki	Terabayashi 1202

Voucher specimens are deposited in the herbarium of Tsumura Central Research Laboratories.

study are listed in Table 1. Reference sources for the scientific names are Dandy (1978) and Ueda (1985, 1986). The trees examined are cultivated and old enough to bear flowers, though trees of *M. biondii* bore no flowers because of their young age. The collection of stem materials was done twice or 3 times a month, obtaining 2 to 3 stems in each collection. For critical comparisons we observed transections from the basal region of current-year stems in *M. acuminata* (L.) L., *M. heptapeta*, *M. quinquepeta*, and *M. tomentosa* Thunb. (= *M. stellata* Maxim.), current-year and one-year-old stems in *M. praecocissima*, and current-year, one-year-old and two-year-old stems in *M. salicifolia* and *M. biondii*. Transections of stems of the second internode of each year were cut as shown in Fig. 1 for the reason that the first internodes are often too short to cut properly. Transections were made at about 20 μm thick, and stained with aceto-methyl green. Before staining some samples were bleached with *Eau de Javell.*

Results

In the seven species examined the following features were observed in common. In middle to late April vegetative buds unfold and new shoots appear. They elongate and show a secondary growth producing xylem and phloem. Three types of cork layer development and cuticle thickening were distinguishable in stems.

The first type found in *Magnolia acuminata*, *M. heptapeta*, *M. quinquepeta* and *M. tomentosa* is characterized by the thin cuticle and the rapid development of a cork layer (Figs. 2–13). A cork cambium composed of radially flattened cells with thin walls usually occurs at several points between the cortex and epidermis and gives

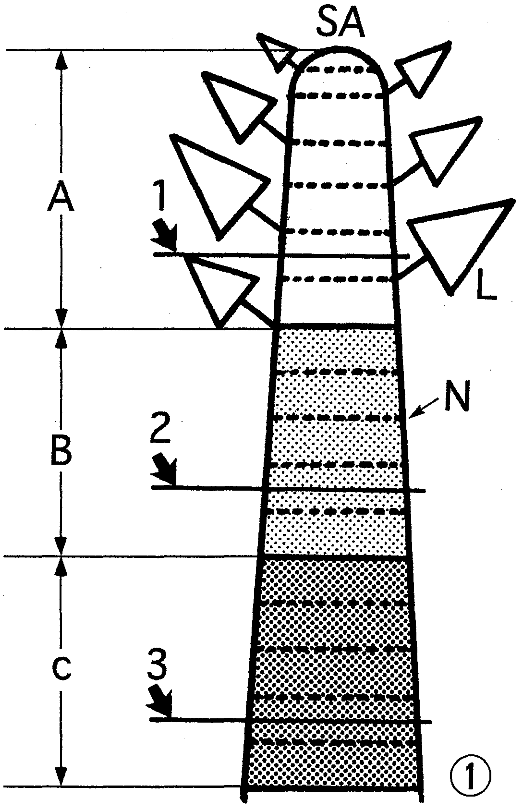
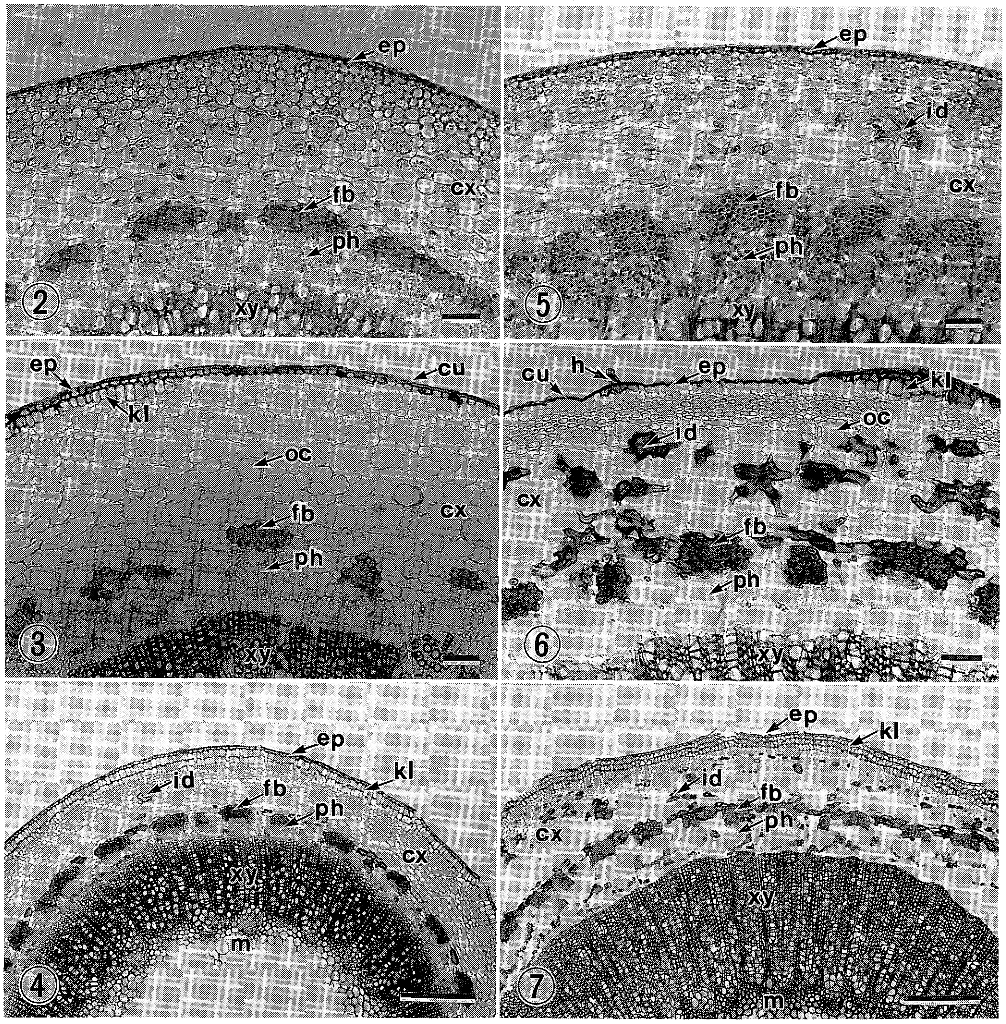


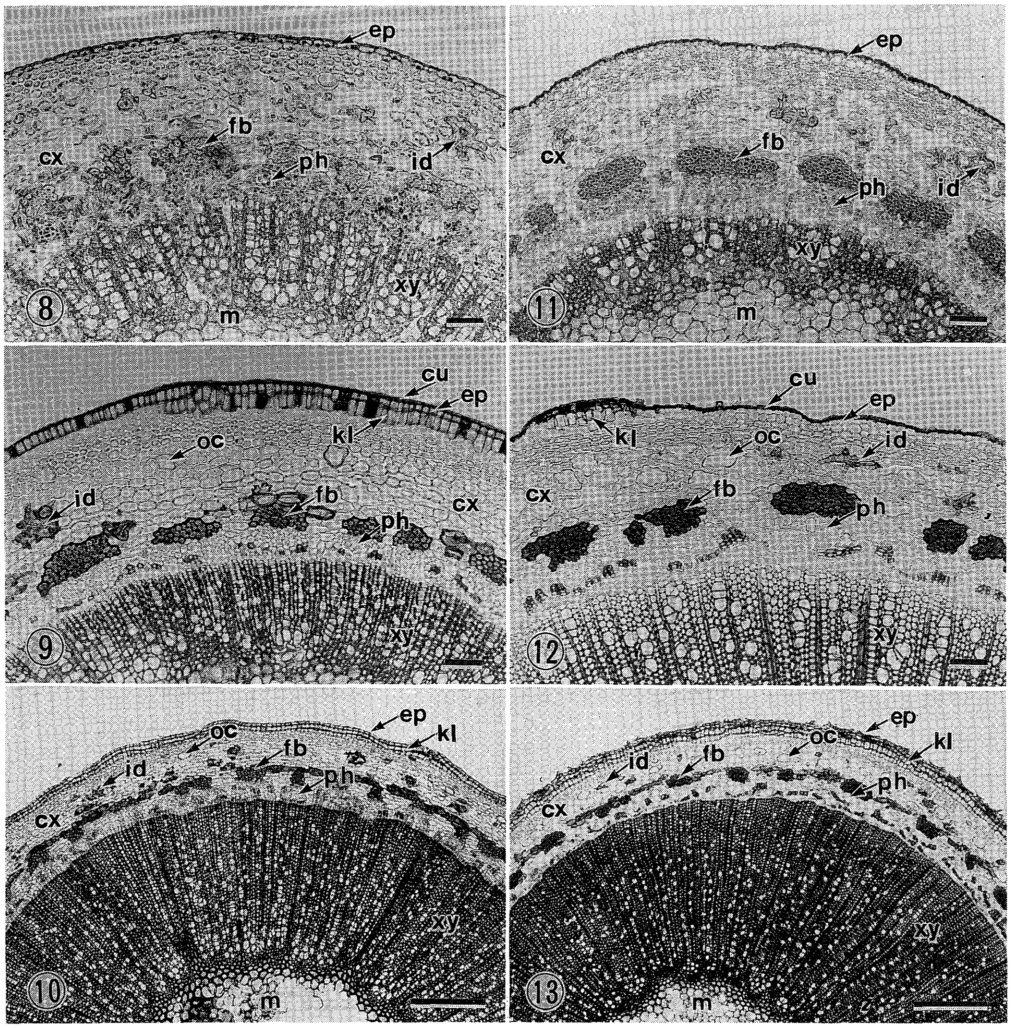
Fig. 1. Diagrammatic illustration of a young *Magnolia* shoot. Arrows (1–3) indicate positions where transections were cut and observed. A: current-year stem; B: one-year-old stem; C: two-year-old stem; L: leaf; N: node; SA: shoot apex.

rise to cork cells outside. Thus a cork layer starts as a discontinuous ring which appears in middle May to early June in *M. heptapeta* and in late May to early July in *M. acuminata*, *M. quinquepeta* and *M. tomentosa* (Figs. 3, 6, 9, 12, 26). In the species of this type, a cork layer develops rather rapidly and more than one layer of cork cells completely encircle the cortex in current-year stems in August (Figs. 4, 7, 10, 13, 27). In new growth the cuticle gradually thickens and remains almost constant in thickness after about 30 days in *M. acuminata* and about 60 days in other species (Fig. 28). In the four species of the first type *M. acuminata* has the thickest cuticle and *M. quinquepeta* has the thinnest cuticle; 4.5–6(–7.5) µm in *M. acuminata*, (2–)2.5–4(–4.5) µm in *M. heptapeta*, (1.5–)2.5–3 µm in *M. quinquepeta* and (2.5–)3–5 µm in *M. tomentosa* (Fig. 28). In August most of current-year stems cease their elongation and are terminated by dormant vegetative or floral buds. The stems are pale green and pubescent (but glabrous only in *M. quinquepeta*) at the terminal region, pale green and pubescent to brown and glabrous at the middle region, and brown and glabrous at the basal region. The change of color and texture of stem occurs earlier on the sunny surface than on the shady surface. The region of stem where cork layers are observed in transections is brown in color. This is due to the brown tannic substances accumulated in cork cells. Also it is often observed that the initiation of cork cells is preceded by the accumulation of brown tannic substances in epidermal cells (Fig. 29).

The second type is observed only in *M. praecocissima* (Figs. 14–17). In this species new shoots arise from vegetative buds in late April as in the species of the first type. A cork layer appears in late May in a few samples, but in early July in most samples. Also in this species a cork layer starts as a discontinuous ring around the stem. In August the cork layer encircles more than half of the cortex in cross sectional view but not completely. It is noteworthy in this species that the cork layer of the current-year stems is partially undifferentiated even at the end of the growing season (late August) (Figs. 14–17, 27). During the growing season of the second year the cork layer of one-year old stems continues to develope and finally encircles completely the cortex (Figs. 17, 27). In the second type the cuticle thickens more slowly and it becomes thicker than that in the first type. The cuticle gradually thickens and attains almost constant thickness of 10–13(–15) µm, in about 100 days after bud unfolding (Fig. 27). In August or



Figs. 2–7. *Magnolia acuminata* and *M. heptapeta*. Figs. 2–4. *M. acuminata*. Transections of current-year stems collected on May 31 (Fig. 2), June 19 (Fig. 3) and July 4 (Fig.4) in 1993. Fig. 2. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 3. Thin cuticle visible, cork layer partially differentiated. Fig. 4. Two layers of cork cells encircling completely the cortex. Figs. 5–7. *M. heptapeta*. Transections of current-year stems collected on April 24 (Fig. 5), May 29 (Fig. 6) and August 9 (Fig. 7) in 1993. Fig. 5. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 6. Thin cuticle visible, cork layer partially differentiated. Fig. 7. Two-three layers of cork cells encircling completely the cortex. Transections bleached and stained with aceto-methyl green in Figs. 3, 4, 6 and 7. cu: cuticle; cx; cortex; ep: epidermis; fb: fiber bundle; h: hair; id:sclerified idioblast; kl: cork layer; m: pith; oc: oil cell; ph: phloem; xy: xylem. Scale bars are 100 µm in Figs. 2, 3, 5 and 6, 500 µm in Figs. 4 and 7.



Figs. 8–13. *Magnolia quinquepeta* and *M. tomentosa*. Figs. 8–10. *M. quinquepeta*. Transections of current-year stems collected on June 2 (Fig. 8), June 19 (Fig. 9) and August 29 (Fig. 10) in 1993. Fig. 8. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 9. Thin cuticle visible, cork layer partially differentiated. Fig. 10. Two-three layers of cork cells encircling completely the cortex. Figs. 11–13. *M. tomentosa*. Transections of current-year stems collected on May 9 (Fig. 11), June 15 (Fig. 12) and August 26 (Fig. 13) in 1993. Fig. 11. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 12. Thin cuticle visible, cork layer partially differentiated. Fig. 13. Two-three layers of cork cells encircling completely the cortex. Transections bleached and stained with aceto-methyl green in Figs. 9, 10, 12 and 13. cu: cuticle; cx; cortex; ep: epidermis; fb: fiber bundle; id:sclerified idioblast; kl: cork layer; m: pith; oc: oil cell; ph: phloem; xy: xylem. Scale bars are 100 μm in Figs. 8, 9, 11 and 12, 500 μm in Figs. 10 and 13.

September most of current-year stems cease their elongation and are terminated by dormant vegetative or floral buds. At the end of the growing season most parts of current-year stems are green or pale green and smooth but partially brown and rough at the basal region. In August of the second year one-year-old stems are mostly brown in color but partially green at the terminal and the middle regions.

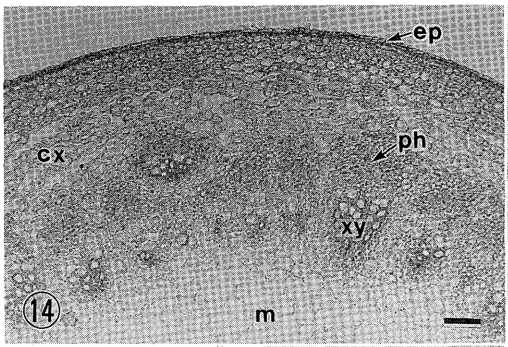
The third type observed in *Magnolia salicifolia* is characterized by the remarkably slow development of a cork layer and the very thick cuticle (Figs. 18–21). In *M. salicifolia* new shoots appear in late April as in the species of the first and the second types. A cork layer is hardly observed in the first year, although the cambium between phloem and xylem is active (Fig. 19). In the second year, a cork layer starts to differentiate outside the cortex. Different from the second type (*M. praecocissima*), complete encircling of the cortex by the cork layer is quite rare in one-year-old stems of the third type (Fig. 20). In the third year, a cork layer is present in every two-year-old stem examined (Figs. 21, 27). In some of these stems, however, the cork layer has not completely encircled the cortex by the end of the growing season of the third year (Fig. 27). In the third type, the cuticle thickens more slowly and becomes thicker than that in the second type. The cuticle becomes (16–)18–24(–28) μm thick approximately 150 days after bud unfolding (Fig. 28). In *M. salicifolia* most of current-year stems cease their elongation and are terminated by dormant vegetative or floral buds in August to September. At the end of the growing season current-year stems are green to pale green in color and smooth in texture. One-year-old stems are still green but partially brown at the basal region. Two-year-old stems are brown as a whole but remain green partially on the shady surface.

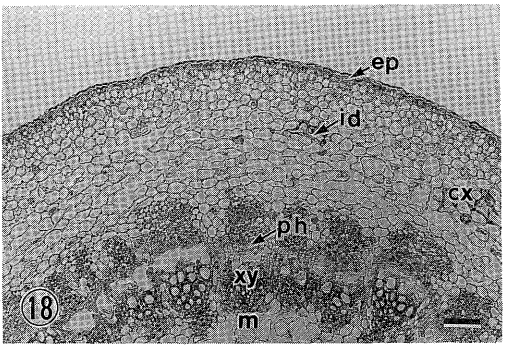
The trees of *M. biondii* were too young for critical comparisons of anatomical features. Preliminary observations in this species showed that it belongs to the third type. A cork layer is not or only a little differentiated in current-year stems (Figs. 22, 23, 27). In the second year, a cork layer develops and partially encircles the cortex (Figs. 24, 27). In the third year, a cork layer completely encircles the cortex (Figs. 25, 27). The cuticle of this species becomes about 12.5–15(–16) μm in thickness (Fig. 28). In August current-year stems are green and smooth throughout their length. At the end of the growing season one-year-old stems are brown on the sunny surface but still green on the shady surface at the basal region as in *M. salicifolia*. Two-year-old stems are brown as a whole but remain green partially on the shady surface.

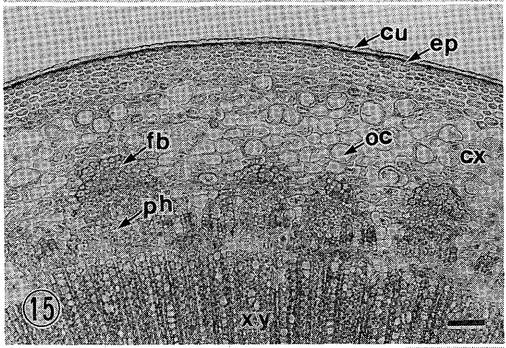
Discussion

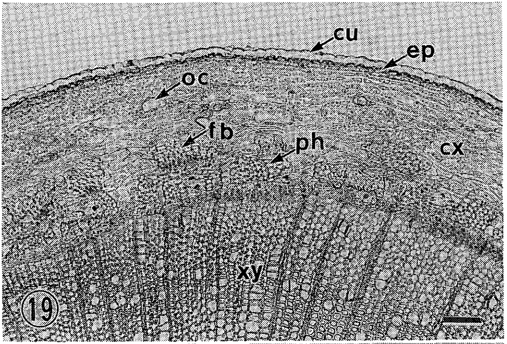
Terabayashi and Okada (1991) reported that the genus *Magnolia* subgenus Yulania showed variation in the development of a cork layer and cuticle thickness in current-year stems bearing floral buds. In *Magnolia acuminata*, *M. heptapeta*, *M. quinquepeta*, and *M. tomentosa* cork layers were present and cuticle was thin. In *M. praecocissima* a cork layer was present or absent and cuticle was thick. In *M. amoena*, *M. biondii* and *M. salicifolia* cork layers were constantly absent and cuticle was thick. The present study on stem development reveals that variation of stem anatomy in the genus *Magnolia* subgenus Yulania (Terabayashi and Okada 1991) is caused by different manners of cork layer development and of cuticle thickening among the species (Stem anatomy was not examined for *M. amoena* in the present study.) As already described above, a cork layer develops rapidly in *M. acuminata*, *M. heptapeta*, *M. quinquepeta* and *M. tomentosa*, slowly in *M. praecocissima*, and remarkably slowly in *M. salicifolia* and *M. biondii*. Cuticle grows less than 7.5 μm in thickness in *M. acuminata*, *M. heptapeta*, *M. quinquepeta* and *M. tomentosa*, but becomes more than 10 μm in *M. praecocissima*, and more than 12.5 μm in *M. salicifolia* and *M. biondii*.

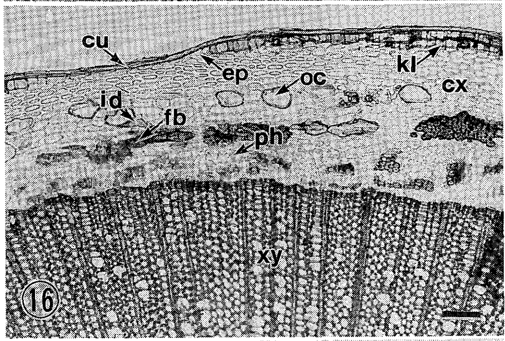
The grouping of genera defined by the stem anatomy does not agree to the classification system proposed by Dandy (1978). The species of Sect. Yulania (*M. heptapeta*.) and of those of Sect. Tulipastrum (*M.

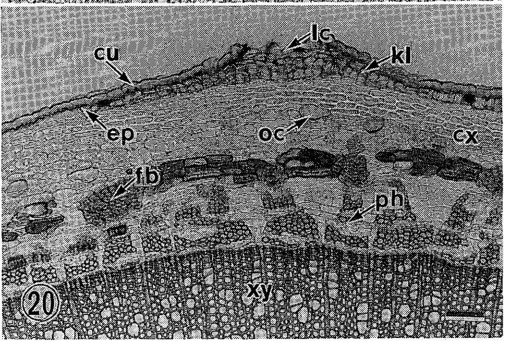


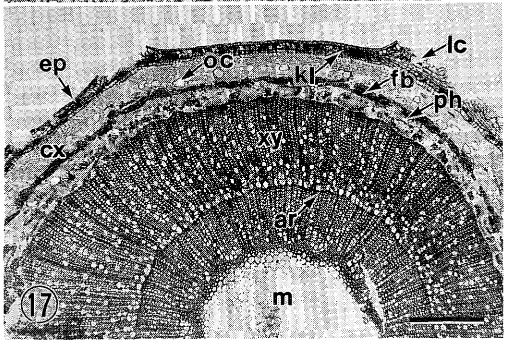


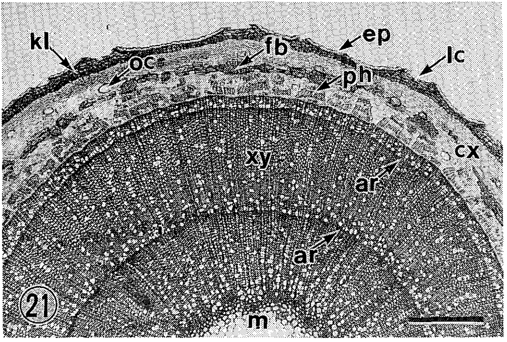


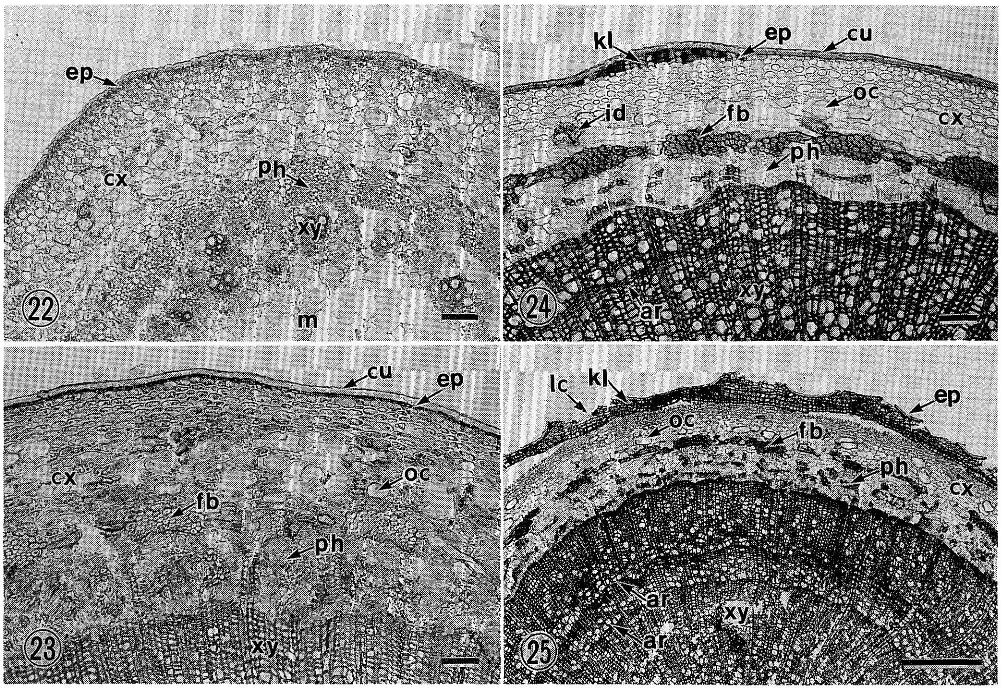












Figs. 22–25. *Magnolia biondii*. Transections of current-year stems collected on April 28 (Fig. 22) and July 25 (Fig. 23) in 1993, one-year-old stems collected on August 3 (Fig. 24) in 1994 and two-year-old stems collected on August 9 (Fig. 25) in 1995. Fig. 22. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 23. Very thick cuticle visible, cork layer not differentiated yet. Fig. 24. Cork layer partially differentiated. Fig. 25. Three-five layers of cork cells encircling completely the cortex, two annual rings observed in xylem. Transections bleached and stained with aceto-methyl green in Figs. 24 and 25. ar: annual ring; cu: cuticle; cx; cortex; ep: epidermis; fb: fiber bundle; id:sclerified idioblast; kl: cork layer; lc: lenticel; m: pith; oc: oil cell; ph: phloem; xy: xylem. Scale bars are 100 μm in Figs. 22, 23 and 24, 500 μm in Fig. 25.

Figs. 14–21. *Magnolia praecocissima* and *M. salicifolia*. Figs. 14–17. *M. praecocissima*. Transections of current-year stems collected on April 27 (Fig. 14), June 27 (Fig. 15) and Aug. 26 (Fig. 16) in 1993 and one-year-old stems collected on July 27 (Fig. 17) in 1994. Fig. 14. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 15. Thick cuticle visible, cork layer not differentiated yet. Fig. 16. Cork layer partially differentiated. Fig. 17. Three-five layers of cork cells encircling completely the cortex, one annual ring observed in xylem. Figs. 18–21. *M. salicifolia*. Transections of current-year stems collected on April 26 (Fig. 18) and July 23 (Fig. 19) in 1993, one-year-old stems collected on August 30 (Fig. 20) in 1994 and two-year-old stems collected on July 23 (Fig. 21) in 1995. Fig. 18. A young stem newly arising from a vegetative bud. Cambium active but cork layers not differentiated yet. Fig. 19. Very thick cuticle visible, cork layer not differentiated yet. Fig. 20. Cork layer partially differentiated. Fig. 21. Three-five layers of cork cells encircling completely the cortex, two annual rings observed in xylem. Transections bleached and stained with aceto-methyl green in Figs. 16, 17, 20 and 21. ar: annual ring; cu: cuticle; cx; cortex; ep: epidermis; fb: fiber bundle; id: sclerified idioblast; kl: cork layer; lc: lenticel; m: pith; oc: oil cell; ph: phloem; xy: xylem. Scale bars are 100 μm in Figs. 14, 15, 16, 18, 19 and 20, 500 μm in Figs. 17 and 21.

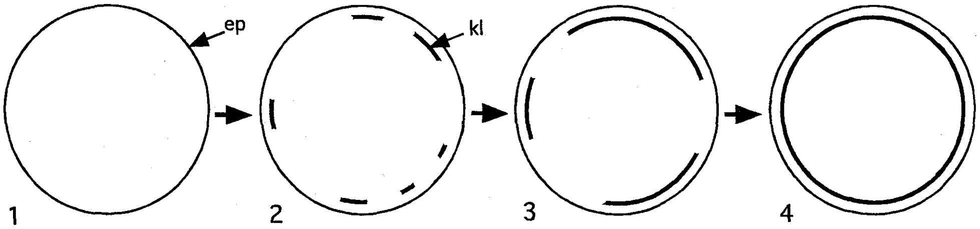


Fig. 26. Diagrammatic illustrations of cork layer development in *Magnolia* stems. 1: Cork layer not differentiated; 2: Cork layer encircling less than half of the cortex; 3: Cork layer encircling more than half of the cortex; 4: Cork layer encircling completely the cortex. ep: epidermis. kl: cork layer.

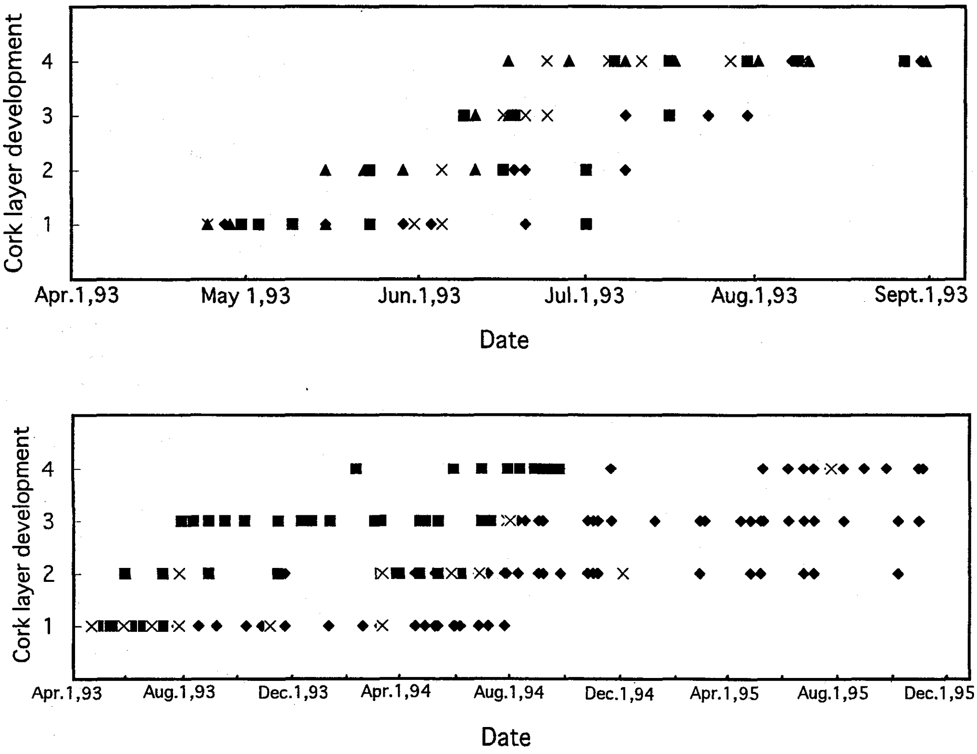


Fig. 27. Cork layer development in *Magnolia* stems examined in 1993–1995. Upper : Cork layer development in *M. acuminata* (×), *M. heptapeta* (▲), *M. quinquepeta* (◆) and *M. tomentosa* (■). Lower: Cork layer development in *M. praecocissima* (■), *M. salicifolia* (◆) and *M. biondii* (×). 1: Cork layer not differentiated; 2: Cork layer encircling less than half of the cortex; 3: Cork layer encircling more than half of the cortex; 4: Cork layer encircling completely the cortex. See Fig. 26.

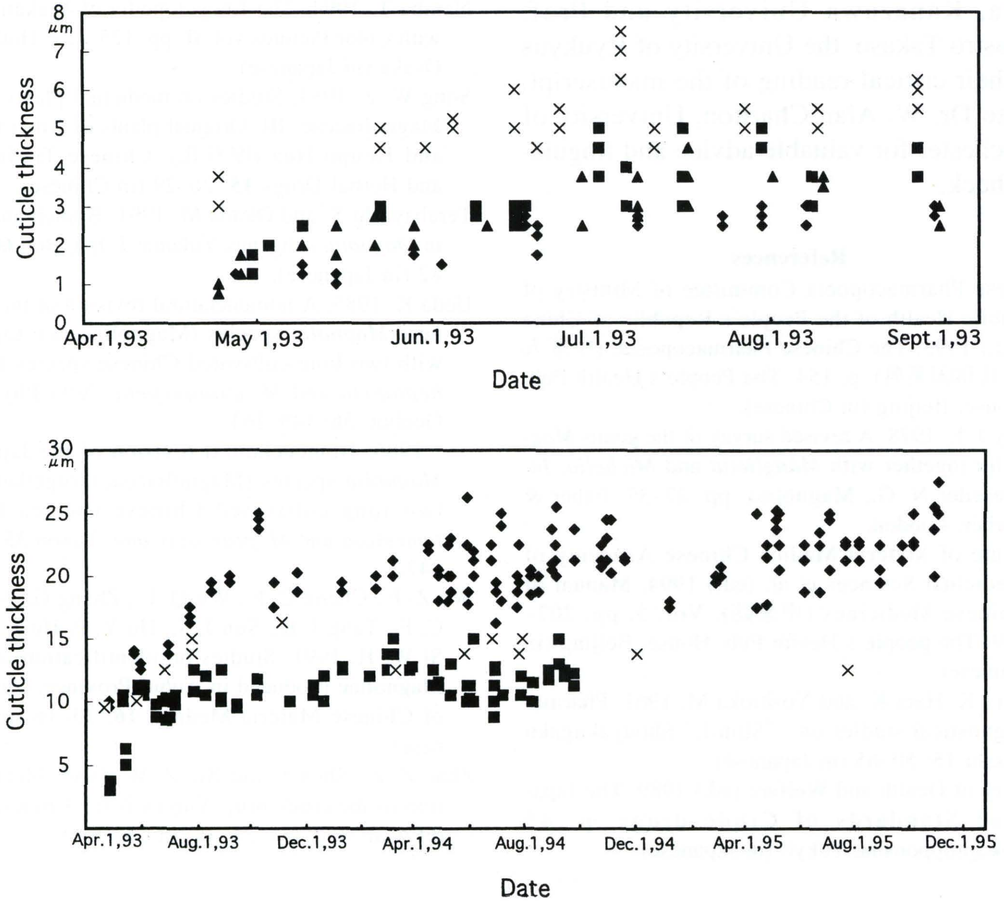


Fig. 28. Cuticle thickening in *Magnolia* stems examined in 1993–1995. Upper: Cuticle thickening in *M. acuminata* (×), *M. heptapeta* (▲), *M. quinquepeta* (◆) and *M. tomentosa* (■). Lower: Cuticle thickening in *M. praecocissima* (■), *M. salicifolia* (◆) and *M. biondii* (×).

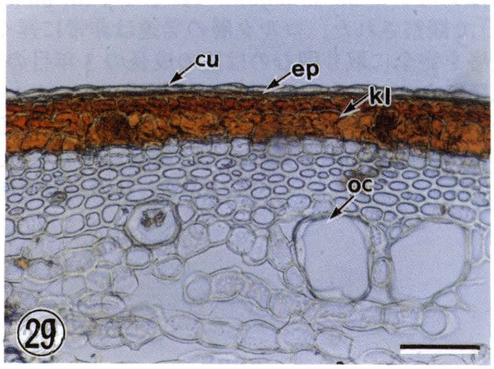


Fig. 29. Brown tannic substances accumulated in epidermal and cork cells in *Magnolie heptapeta*. cu: cuticle; ep: epidermis; kl: cork layer; oc: oil cell. Scale bar is 100 μm.

acuminata and *M. quinquepeta*) show the first type. Sect. Buergeria is diverse in the stem anatomy: *M. tomentosa* is of the first type, *M. praecocissima* is of the second and *M. salicifolia* and *M. biondii* are of the third.

It is noteworthy that in the species with thin cuticle a cork layer develops rapidly (the first type), while in those with thick cuticle a cork layer develops slowly (the second and the third types). It is a future project to make clear the ecological or plant geographical implications of the different types of stem anatomy described here.

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References

Chinese Pharmacopoeia Committee of Ministry of Public Health of the People's Republic of China (ed.) 1995. The Chinese Pharmacopoeia, (中華人民共和国薬典). p. 154. The People's Health Pub. House, Beijing (in Chinese).

Dandy J. E. 1978. A revised survey of the genus *Magnolia* together with *Manglietia* and *Michelia*. *In*: Treseder N. G., Magnolias. pp. 27–37. Faber & Farber, London.

Institute of Materia Medica Chinese Academy of Medicinal Sciences et al. (ed.) 1994. Manual of Chinese Medicines (中薬誌). Vol. 5, pp. 207–219. The people's Health Pub. House, Beijing (in Chinese).

Kimura K. Hata K. and Yoshioka M. 1961. Pharmacognostical studies on "Shin-i." Shouyakugaku Zasshi **15**: 50–65 (in Japanese).

Minstry of Health and Welfare (ed.) 1989. The Japanese Standards of Crude drugs. p. 45 Yakujinippousha, Tokyo (in Japanese).

Namba T. 1994. The Encyclopedia of Wakan-Yaku with Color Pictures vol. II. pp. 125–127. Hoikusha, Osaka (in Japanese).

Song W. Z. 1984. Studies on medicinal plants of the Magnoliaceae. III. Original plants of Xinyi (辛夷) and Houpo-Hua (厚朴花). Chinese Traditional and Herbal Drugs **15**: 26–29 (in Chinese).

Terabayashi S. and Okada M. 1991. Branch anatomy in *Magnolia* subgenus Yulania. J. Jpn. Bot. **66**: 76–82 (in Japanese).

Ueda K. 1985. A nomenclatural revision of the Japanese *Magnolia* species (Magnoliaceae), together with two long-cultivated Chinese species III. *M. heptapeta* and *M. quinquepeta*. Acta Phytotax. Geobot. **36**: 149–161.

——1986. Nomenclatural revision of the Japanese *Magnolia* species (Magnoliaceae), together with two long-cultivated Chinese species II. *M. tomentosa* and *M. praecocissima*. Taxon **35**: 344–347.

Wu Z. F., Cheng C. F., Wu Q. L., Zhang G. W., Wu C. F., Tang J. R., Sun J. S., Hu Y. P, Hu J. J. and Si W. H. 1991. Studies on identification of Flos Magnoliae produced in Anhui Province, China. J. of Chinese Materia Medica. **16**: 13–16 (in Chinese).

Zhao Z. Z., Shen J. and Xie Z. W. 1986. Identification of the crude drug Xinyi (辛夷). Chinese Traditional Med. **(3)**: 34–38 (in Chinese).

寺林　進, 樋口正視, 岡田　稔: モクレン属ハクモクレン亜属の茎におけるコルク層とクチクラの発達

モクレン属ハクモクレン亜属7種について，茎におけるコルク層とクチクラの発達を比較観察した．その結果，3タイプが認められた．第一のタイプは，キモクレン *Magnolia acuminata*，ハクモクレン *M. heptapeta*，シモクレン *M. quinquepeta*，シデコブシ *M. tomentosa* で観察されたもので，コルク層の発達は速く，当年茎において夏までに皮層を完全に取り囲み，クチクラは薄いのが特徴である．第二のタイプはコブシ *M. praecocissima* に見られたもので，コルク層の発達は遅く，皮層を完全に取り囲むのは茎の成長の2年目の春以降である．第一のタイプに比しクチクラは厚い．第三のタイプはタムシバ *M. salicifolia*（おそらく *M. biondii* も）で観察された．コルク層の発達は非常に遅く，皮層を完全に取り囲むのは茎の成長の3年目の春以降であり，中には3年目の成長期においてもコルク層が部分的にしか分化していないものもある．クチクラは3つのタイプの中では最も厚くなる．

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